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Reelin Through the Years: From Brain Development to Inflammation

Anna Alexander[1,2], **Joachim Herz, M.D.**[1,2,3,4], **Laurent Calvier, Ph.D.**[1,2]

[1]Department of Molecular Genetics, University of Texas (UT) Southwestern Medical Center, Dallas, USA.

[2]Center for Translational Neurodegeneration Research, UT Southwestern Medical Center, Dallas, USA.

[3]Department of Neuroscience, UT Southwestern Medical Center, Dallas, USA.

[4]Department of Neurology and Neurotherapeutics, UT Southwestern Medical Center, Dallas, USA.

Abstract

Reelin was originally identified as a regulator of neuronal migration and synaptic function, but its non-neuronal functions have received far less attention. Reelin participates in organ development and physiological functions in various tissues, but it is also dysregulated in some diseases.

In the cardiovascular system, Reelin is abundant in the blood where it contributes to platelet adhesion, coagulation, as well as vascular adhesion and permeability to leukocytes. It is a pro-inflammatory and pro-thrombotic factor with important implications for auto-inflammatory and auto-immune diseases such as multiple sclerosis, Alzheimer's disease, arthritis, atherosclerosis, or cancer.

Mechanistically, Reelin is a large secreted glycoprotein that binds to several membrane receptors including ApoER2, VLDLR, integrins, and ephrins. Reelin signaling depends on the cell type but mostly involves phosphorylation of NF-κB, PI3K, AKT, or JAK/STAT.

This review focuses on non-neuronal functions and the therapeutic potential of Reelin while highlighting secretion, signaling, and functional similarities between cell types.

Keywords

Reelin; ApoER2; VLDR; NF-κB; PI3K; AKT; JAK; STAT; liver; vascular system; coagulation; lymphatic circulation; immune system; intestine; kidney; cancer; fibrosis; inflammation; leukocyte; multiple sclerosis; Alzheimer's disease; atherosclerosis; arthritis

*Corresponding author: Laurent Calvier, Ph.D. Molecular Genetics, UT Southwestern Medical Center, 5323 Harry Hines Blvd. Dallas, TX 75390-9046, calvier.laurent@gmail.com.

AUTHOR CONTRIBUTIONS

A.A. wrote the manuscript, J.H and L.C. have revised it for interpretation and content.

DECLARATION OF INTERESTS

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1) INTRODUCTION

Reelin is a large extracellular glycoprotein initially known as a regulator of neuronal migration whose secretion is critical for development and migration, especially in the cortex and hippocampus[1–3]. During embryonic development, Reelin is mainly secreted by Cajal-Retzius cells and promotes neuroblast migration and development of layer-specific connections in the hippocampus and entorhinal cortex[1,4–7]. In the adult brain, Reelin is mainly secreted by a subset of cortical GABA-ergic interneurons[5,8,9], cerebellar granule cells and hippocampal interneurons[10–12] and modulates synaptic plasticity[6,13], migration of neuroblasts[14], as well as dendrite[15] and dendritic spine[16] formation. Reelin has been implicated in a wide range of neurodegenerative diseases, such as Alzheimer's disease (AD), cognitive disorders, such as autism spectrum disorder, and neuropsychiatric disorders, such as schizophrenia and bipolar disorder[12,17–22].

In 1951, a mutant mouse model showing a characteristic "reeling" gait was documented for the first time and named reeler mice[23]. This behavior was later attributed to a disruption in the layered structure of the brain[24,25] due to the absence of Reelin expression[2]. This model has been largely used to study various aspects of brain development, including neuronal migration, synapse formation, and neurodevelopmental disorders such as autism and schizophrenia. Interestingly, the neurons retain their morphology with intact cellular connectivity despite their disorganized positioning, and comprehensive studies suggest that a layer loss only leads to defects in sensory processing by reeler mice while cortical layers are not an essential for basic perception and cognition[26]. In 1995, the *RELN* gene was identified on chromosome 7q22[2] and the anti-Reelin mouse monoclonal antibody CR-50 was engineered with the remarkable ability to block Reelin's function[7,27]. CR-50's mechanism is through binding to the N-terminal (N-t) region which in turn blocks multimerization of Reelin, a prerequisite to Reelin's biological activity[27]. In 1999, the two main receptors of Reelin, Apolipoprotein E receptor 2 (ApoER2, also known as LRP8) and very low density lipoprotein receptor (VLDLR), were discovered through the presence of the Dab1-binding NPxY motif in their cytoplasmic domains and the finding that Apoer2/Vldr double KO mice phenocopy the reeler (and scrambler) mice[28,29].

Reelin's structure (Figure 1) is comprised of a signal peptide followed by an F-spondin-like domain, eight Reelin repeats that have an epidermal growth factor (EGF)-like cysteine pattern dividing each repeat into two subdomains (A and B), and a positively charged C-terminus[30]. Originally, two cleavage points were identified: one located between Reelin repeats 2 and 3 and one between repeats 6 and 7[31,32]. Recently, the N-terminal cleavage site was more precisely identified at the beginning of repeat 3 between Pro-1244 and Ala-1245, which is supported in both in vivo and in vitro studies[33–35]. Three fragments are produced: The N-terminal, central, and C-terminal fragments; however, these two cleavage events occur independently, meaning the central fragment can be attached to either terminus upon proteolytic cleavage. Of the three fragments produced, the central fragment containing repeats 3 through 6 is the active one as the receptor binding domain for canonical Reelin receptors is located on Reelin repeats 5 and 6[32,36].

Since its initial discovery, Reelin's main studies have been focused on its beneficial function in nervous system, but over the past two decades, Reelin has been related to various non-neuronal functions, suggesting that Reelin may have implications in cancer propagation, coagulation deficits, and endothelial dysfunction in chronic inflammatory diseases[37–40] (Figure 2). Thus, Reelin has proven to have a pleiotropic nature, with beneficial effects in the brain and diverse effects in the periphery. The goal of this review is to encompass the wide versatility of responses to Reelin in the peripheral systems. The reader should grasp the benefits as well as detriments of abnormal Reelin expression in mouse models, human patients, and various cell lines.

2) REELIN REGULATION AND PATHWAYS

Reelin pathways have been primarily studied in neurons, as outlined below, and confirmed in different cell types. Reelin has two major membrane receptors, ApoER2 and VLDLR, both of which belong to the low-density lipoprotein receptor (LDLR) family[28–30,41–43]. These receptors have an Asn-Pro-X-Tyr (NPxY) motif on their cytoplasmic tails, a common feature of the LDLR family[29,44]. Upon Reelin binding the extracellular surface of these receptors, the NPxY domain causes the recruitment of Disabled-1 (Dab1), the intracellular adaptor protein that facilitates Reelin signaling[29,44,45]. This induces tyrosine phosphorylation of Dab1 by Src-family tyrosine kinases (SFKs), specifically Fyn and Src, which are activated by the clustering of receptors upon Reelin signaling[46–48]. Interestingly, the reeler phenotype can also be replicated via double knock-out of ApoER2 and VLDLR, as well as through loss of Dab1, known as the scrambler mouse model, demonstrating the importance of these receptors on Reelin processing[29,45,49,50]. Reelin not only cues the phosphorylation of Dab1, but it also signals for its degradation through polyubiquitination[51]. After the initial Reelin-induced Dab1 phosphorylation event, various pathways may become active underlying Reelin's diverse effects at the molecular level.

One major Reelin-inducible effect is activation of phosphatidylinositol 3-kinase (PI3K) by its regulatory subunit p85α, which activates the serine/threonine kinase Akt and inhibits glycogen synthase kinase 3β (GSK-3β), an enzyme responsible for tau phosphorylation[52,53]. p85α forms a complex with the tyrosine-phosphorylated Dab1 in order to activate PI3K, stimulating downstream effectors Akt and GSK-3β via phosphorylation of specific serine residues[52,53]. PI3K and Akt are crucial components in cortical development, and Akt stimulates the canonical mammalian target of rapamycin (mTOR) pathway which controls dendritic growth and branching[54]. This involves the protein complexes mTORC1 and mTORC2, as well as S6 kinase 1 (S6K1), which modulates protein synthesis and polarizes actin organization within the cytoskeleton[54,55]. Akt also influences cancer through induction of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) through extensive crosstalk with mTOR[56].

Others transmembrane proteins and potential receptors studied in reference to Reelin include integrins and the amyloid precursor protein (APP). Integrins are transmembrane receptors that participate in neuronal migration and outgrowth by influencing neuronal adhesion to the extracellular matrix[57–61]. Reelin has been shown to directly bind the α3β1 integrin and activate α5β1, suggesting alternate pathways for neuronal migration and positioning[59,60].

APP is a type I transmembrane protein that activates the central Reelin fragment and interacts with the integrin α3β1 in order to promote neurite outgrowth[62,63]. However, APP is processed to form biologically-active amyloid-beta peptides that can accumulate when unregulated and result in amyloid plaques as characterized in Alzheimer's disease[63–66]. Aberrant APP processing also coincides with Reelin depletion in AD brains[21]. Furthermore, APP overexpression in mammalian cortical neurons results in reduction of Dab1 tyrosine phosphorylation, suggesting that APP may quench Reelin signaling by interfering with the canonical Reelin pathway potentially by altering stoichiometry of Reelin coreceptors, but direct evidence of this potential competition is missing [67].

Other Reelin pathways that have been noted in neurons include Crk-family proteins, LIM kinase 1 (LIMK1), and ephrins. After initial Dab1 phosphorylation, Crk-family proteins induce tyrosine phosphorylation of C3G in order to activate Rap1, part of the Ras subfamily of GTPases, to regulate neuronal polarization during radial migration of neurons in the developing cortical plate[68,69]. Reelin not only directs migrating neurons but also stabilizes their cytoskeleton in the marginal zone through LIM kinase 1 and cofilin intracellular signaling[70–73]. Although the mechanism is unknown, Reelin also reportedly binds both ephrin B ligands and Eph B receptors[74–77]. One suggested mechanism is that ligands ephrin B2 and ephrin B3 influence cortical layering by recruiting and activating SFKs within ApoER2 and VLDLR receptor clusters during canonical Reelin signaling[76]. This finding is arguably dependent on mouse background, as the reeler-like phenotype was not seen in similar models by Pohlkamp et. al. in 2016. However, Reelin and ephrins have some interaction as seen by the lack of surviving ephrin B3 and Reelin double knockout mice[77].

Research in adult mouse brains connects Reelin, through activated SFKs, to proteins that regulate long term potentiation, N-methyl-D-aspartic acid receptor (NMDAR) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR)[6,13]. Acute Reelin exposure allows for NMDAR activation via phosphorylation of NR2 subunits by Src, whereas longer Reelin exposure increases AMPAR at synaptic sites through PI3K[13,78,79].

Some of these canonical (ApoER2-dependent) pathways have been validated in non-neuronal cells as detailed in each section of this review, but most of the non-canonical ones remain yet to be confirmed.

3) REELIN AND THE LIVER

The liver, specifically hepatic stellate cells, are believed to be the main source of Reelin outside of the brain, and Reelin secretion in these cells has been documented after liver injury[80–84]. Hepatic stellate cells have the ability to transdifferentiate into collagen-producing myofibroblasts upon activation during liver fibrosis[85–87]. Stellate cells amplify liver inflammation[88] by releasing various inflammatory molecules and promoting leukocyte chemotaxis and adherence[89]. Additionally, these cells respond to many inflammatory signals. They express different receptors such as toll-like receptors, IL-receptors, Fc receptors, TNFR, CXCR3, intracellular adhesion molecule (ICAM)-1, the C5a receptor (CD88)[85,88,89] and directly respond to diverse immunological triggers such as cytokines including TNFα, IFNγ, and IL-6[85,89]. While stellate cell response to these various cytokines

is well understood, it is unknown if these cytokine responses also lead to increased Reelin secretion.

Activation of hepatic stellate cells in liver disease results in Reelin production.

In patients with liver diseases such as fibrosis or cirrhosis, activated hepatic stellate cells increase Reelin expression[80,81], although secretion into the circulation has not yet been established. Immunohistochemistry of liver samples from patients with Hepatitis C Virus (HCV)-related chronic liver disease showed that Reelin was coexpressed with CRBP-1, a marker used to detect activated stellate cells in liver disease[81]. Both Reelin and CRBP-1 positive stellate cells showed direct correlation to liver fibrosis progression, increasing with worsening disease state[81]. However, Reelin was not correlated with alpha-SMA, another marker for hepatic stellate cells, and the authors suggest that Reelin should not be regarded as a marker of hepatic stellate cells/myofibroblasts differentiation but rather as a functional protein expressed during some phases of liver fibrosis. Hepatic Reelin appears to be a product of the disease and not an inducer, yet the potential of plasma Reelin as a biomarker remains to be evaluated. Beyond liver fibrosis or cirrhosis, studying if plasma Reelin is also secreted by the liver may be relevant for atherosclerosis to evaluate the disease activity.

4) REELIN AND THE VASCULAR SYSTEM

Outside of the central nervous system (CNS), the source for circulating Reelin is unclear, but it is suspected that it is mainly produced by hepatic stellate cells and released into the bloodstream[82]. Other peripheral sources where Reelin expression has been detected include the kidney, adrenal medulla, lymphatic vessels, small intestine, submandibular gland, cartilage, and bone[83,90–96]. Nevertheless, Reelin is abundant in blood as seen from the analysis of plasma and serum in both mice and humans, therefore a role for this circulating protein on the vasculature has been hypothesized. Since Reelin is beneficial in the brain, a similar protective function was envisaged for the vascular system. However, ultimately the role of Reelin in the vasculature appears to be more complex.

In 2016, Ding et al. demonstrated both whole body or circulation-specific depletion of Reelin greatly reduced disease pathology in the low-density lipoprotein receptor (LDLR) deficiency mouse model of atherosclerosis[38]. Lesion area size of the aorta was markedly reduced in both whole body and circulation-specific mouse models and the reduction of monocyte infiltration into these lesions correlated with a reduction of vascular adhesion marker protein expression on the endothelium, such as vascular cell adhesin molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1)[38]. To determine the role of Reelin for leukocyte recruitment, intravital microscopy was performed on WT and Reelin conditional knockout (cKO) healthy mice to follow leukocyte rolling on the vessel wall[38]. When compared to controls, leukocyte velocity was increased while the number of rolling leukocytes decreased in Reelin cKO mice, indicating less adhesion to the endothelium. This suggests that Reelin promotes inflammatory cell recruitment and inflammation by increasing the expression of leukocyte-endothelial adhesion proteins on the endothelial surface[38].

Further *in vitro* experiments on human aortic endothelial cells have demonstrated that Reelin binds to its receptor ApoER2 to activate a phosphorylation cascade through Dab-2, PI3K,

Akt and importantly NF-κB[38,97]. Activation of NF-κB directly promotes the expression of adhesion molecules, such as VCAM-1, ICAM-1 and E-selectin. In addition, Reelin also reduces endothelial NOS (eNOS), a naturally occurring anti-adhesion molecule with potent vasodilation properties that manages systemic blood pressure[38,98], and promotes vascular smooth muscle cell proliferation via STAT3 phosphorylation[97]. It appears that Reelin is implicated as a ligand for multiple pathways regarding atherosclerosis. Double knockouts for ApoE and either Stabilin-1 or Stabilin-2 showed reduced aortic plaque development when compared to lone ApoE knockout (KO) mice[99]. Upon proteomic analysis of these mice, Reelin was found to be a novel ligand for the Stabilins[99]. This is the first instance reporting Stabilins with a role in atherosclerosis and Reelin as a ligand of Stabilins.

Based on these previous observations, anti-Reelin strategies have been devised to dampen leukocyte extravasation. In the atherosclerosis-prone LDLR KO model fed with a high cholesterol diet, prophylactic Reelin depletion by anti-Reelin antibody (CR-50) or antisense oligonucleotide (ASO) decreases plaque formation in the aorta[97]. These findings suggest that Reelin inhibition may provide a novel therapeutic approach to counteract leukocyte or monocyte adhesion as well as extravasation and inhibit the progression of atherosclerosis.

5) REELIN AND NEUROINFLAMMATION

The results on atherosclerosis have raised the intriguing question of how the organism “reuses” a neuron guidance molecule (Reelin), that is crucial for brain development to pave the way for leukocyte extravasation and guide them to the perivascular space during inflammation. This new paradigm on the role of guidance molecules across different functions and cell types has been further investigated in the neurovascular breakdown that occurs during neuroinflammation. In multiple sclerosis (MS), chronic inflammation allows monocytes to infiltrate the nervous system resulting in demyelination and eventual paralysis[100,101]. Unfortunately, medications that mitigate leukocyte infiltration can leave the patient vulnerable to opportunistic infections[102–104]. Thus, other therapeutic options must be explored[105].

As highlighted previously, expression of inflammatory mediators (E-selectin, ICAM-1, and VCAM-1) in human endothelial cells is increased by Reelin through its receptor ApoER2 and NF-κB signaling, both known to regulate endothelial homeostasis[38,106]. Accordingly, Reelin serum concentrations from MS patients were raised during relapse phases and decreased to control levels during remission phases[37]. This suggested that the amount of circulating Reelin could directly correlate to disease phase and severity in MS. Thus, the prophylactic and therapeutic potential of circulating Reelin depletion via CR-50 antibody treatment was tested in the mouse model of experimental autoimmune encephalomyelitis (EAE) and the results were further validated in Reelin cKO mice[37]. Removal of plasma Reelin by either approach protected against neuroinflammation and largely abolished the neurological consequences by reducing endothelial expression of adhesion proteins (E-selectin and ICAM-1) and consequently reducing immune cell (monocytes, B cells, T helper cells and T cells) accumulation in the CNS[37]. Depletion of Reelin also protected mice from paralysis by inhibiting disease progression, as seen with decreased EAE clinical scores

and lower weight loss when compared to controls[37]. Importantly, peripheral treatment with CR-50 did not deplete or alter Reelin function in the CNS[37].

These results were further confirmed in a second study focused on the Reelin receptor ApoER2. After induction of EAE in mice with either *Apoer2* KO or loss-of-function mutation (*Apoer2* EIG), neuroinflammation and paralysis were largely abolished[107]. There was also downregulation of the vascular adhesion proteins E-selectin and ICAM-1 in the spinal cord, thus ApoER2 deficiency causes dampening of neuroinflammatory signals[107]. This was further supported using intravital microscopy on both Apoer2 models, demonstrating leukocyte adhesion to the endothelium was reduced in the Apoer2 mutants.

All these models are recapitulated in Figure 3, illustrating that Reelin or ApoER2 disruption reduces the expression of adhesion molecules, infiltration of leukocytes, and paralysis. This suggests a role for circulating Reelin in neuroinflammatory diseases where the blood-brain barrier is compromised, like multiple sclerosis (MS) and Alzheimer's disease (AD).

Genome-wide association studies of APOE-ε4 homozygous individuals, a known genetic risk factor for AD[108,109], led to the finding that *DAB1-RELN* single nucleotide polymorphisms were also associated with AD[110]. Indeed, the *RELN* locus is genetically associated with not only AD but various neuropsychiatric disorders including schizophrenia, bipolar disorder, and autism spectrum disorder[111–113]. Although this data shows the pathway as a whole influencing AD incidence, it does not on its own provide mechanistic insight into proper therapeutic intervention. The main features of AD are β-amyloid (Aβ) plaque deposition[114–118], neurofibrillary tau tangles[114,115], and infiltration of inflammatory cells, a more recent hallmark gaining increasing attention[119–121]. Although initial monocyte infiltration bolsters Aβ clearance, a sustained inflammatory environment is detrimental and hastens AD progression[122,123]. Since circulating Reelin promotes neuroinflammation, it poses as a new therapeutic target for AD. Although this has not been tested directly in the AD context as of yet, Reelin depletion decreases blood-brain barrier permeability to leukocytes, thus dampening neuroinflammation[37,107]. Future studies are required and should include testing plasma Reelin levels in AD patients to determine the translational relevance for human neuroinflammation.

6) REELIN AND ARTHRITIS

Arthritis is a leading cause of pain and disability globally, affecting people of all ages. This term regroups different diseases and conditions all causing degradation in the joints and surrounding tissues. There are several types of arthritis that can be differentiated between inflammatory forms featuring warm or swollen joints, such as rheumatoid arthritis or ankylosis spondylitis, and non-inflammatory forms such as osteoarthritis, the most common type of arthritis. Due to the important burden of arthritis on the society and patients, many studies have been conducted to identified genetic causes and biomarkers, among some of which Reelin has been identified[124–128].

First in a very small arthritis cohort, patients with rheumatoid arthritis presented increased Reelin expression in serum and synovial fluid compared to patients with osteoarthritis[125]. In

the same study Reelin mRNA expression was detected in fibroblast-like synoviocytes. In a second larger study, Reelin protein expression was found also differentially expressed in the serum of rheumatoid patients[124]. In a different form of inflammatory arthritis, Reelin variant p.(Ser2486Gly) was identified in a family with ankylosis spondylitis, associating for the first time a Reelin SNP mutation in the ApoER2 binding region to a form of arthritis[126].

Although osteoarthritis has traditionally been classified as a noninflammatory arthritis, it also relies on inflammation of the synovial membrane and recruitment of immune processes, attesting an ongoing low grade chronic inflammation[129–131]. In a rat model for osteoarthritis, Reelin mRNA expression was elevated in ipsilateral cartilage compared to sham-operated cartilage[127]. In addition, Reelin mRNA expression was also increased in differentiating chondrocyte culture.

Taken together, these results suggest that Reelin is associated with several forms of arthritis, but the underlying mechanism remains unknown. Given the role described for this protein in endothelial dysfunction and infiltration of leukocytes[37,38,97,102,107] and the importance of leukocyte infiltration in arthritis, future studies should be designed to test this mechanism in inflammatory arthritis models.

7) REELIN AND COAGULATION

Platelets are also suspected to be a source of production and storage of Reelin[132]. Therefore, its role in coagulation has been recently investigated. Platelets are normally dormant in the vasculature unless there is vessel wall injury. This damage triggers accumulation of platelets that adhere to one another in order to seal the vessel via hemostasis[133]. Increased adhesion of leukocytes is a result of Reelin stimulation[37,97,107]. Therefore, Reelin stimulation could bolster repair of injured vessels by increasing platelet adhesion. However, this can be problematic for patients with hypercoagulable disorders in which there is excessive clot formation, thus Reelin ablation could be beneficial for thrombosis therapies.

Reelin ablation reduces thrombus formation.

Reeler mice are noted as coagulation deficient and thus have inhibited thrombosis and hemostasis. *In vivo*, reeler mice had no incidence of arterial thrombosis 40 minutes after $FeCl_3$ injury to mesenteric arterioles, whereas 100% of wild type mice had complete vessel occlusion by thrombus formation at this point[134]. Furthermore, this coagulation deficit was recapitulated in WT mice treated with the anti-Reelin antibody CR-50[134]. Further, in a tail bleeding assay, reeler mice bled for significantly longer periods of time and were more prone to rebleeding than heterozygotes or wild-type controls[39]. This blunted hemostasis is a result of abnormal fibrin clot formation indicative of deficient thrombin generation and altered prothrombin cleavage during blood coagulation efforts[39]. Thus, Reelin has a role in thrombus formation further extending its role in adhesion in peripheral systems. The evidence from these studies points to selective ablation of Reelin in the circulatory system as a therapeutic target for diseases such as prothrombin gene mutations or dysfibrinogenemia, as well as acquired hypercoagulable disorders, such as heart attacks or strokes.

Reelin's receptors, APP and ApoER2, influence fibrinogen adhesion.

Multiple pathways have been studied referencing Reelin's influence on coagulation. Thrombus formation occurs when platelets activate adhesion-signaling receptors, like the glycoprotein (GP) receptors GPVI and GPIb-IX-V, that interact with various extracellular matrix proteins, such as collagen, fibrinogen, laminin and von Willebrand factor[133,135]. When investigating canonical Reelin receptors, a link between Amyloid precursor protein (APP) and ApoER2 was found. Extracellular Reelin treatment on wild-type platelets increased platelet adhesion to fibrinogen[134,136], but not in APP deficient ($App^{-/-}$) and ApoER2 deficient ($Lrp8^{-/-}$) platelets[134]. To explore thrombus formation in the circulation, whole blood was used *ex vivo* from APP and ApoER2 deficient mice. Without Reelin treatment, less thrombi formation and surface coverage was seen at high shear conditions for blood from $App^{-/-}$ mice, similar to that of reeler blood, yet surprisingly blood from $Lrp8^{-/-}$ mice formed thrombi similar to that of WT blood[134]. However, in the same experiment, extracellular Reelin treatment rescued $App^{-/-}$ phenotype suggesting that APP alone is not sufficient for Reelin signaling in platelets. The authors suggest that APP is a receptor influencing Reelin's role in platelet adhesion, although APP and ApoER2 appear to share some functions within this phenomenon[134]. Of note, VLDLR, another Reelin receptor, showed to have no effect on platelet adhesion[134].

Glycoprotein VI is a platelet receptor for Reelin influencing collagen adhesion.

Other coagulation mechanisms for Reelin have also been proposed to explain its influence on platelet adhesion. As stated previously, exogenous Reelin treatment increases platelet adhesion to fibrinogen, but the effect of Reelin on collagen adhesion remained unknown. Recent work demonstrated WT-platelet adhesion to collagen was unaltered by Reelin treatment; however, reeler platelet adhesion to collagen was lower than WT-platelet adhesion[136]. This decreased adhesion was rescued by recombinant Reelin treatment, promoting high levels of collagen adhesion indistinguishable from controls[136]. This study further suggested that Reelin may stimulate Glycoprotein VI (GPVI), a major collagen receptor found on the platelet surface[135,137]. GPVI induces tyrosine phosphorylation on target proteins, including PLC-γ2 and Syk, which stimulates platelet adhesion, cytoskeletal reorganization and clot retraction[133,136]. Reelin's role in this pathway was further supported *in vivo*. Antibody-induced inhibition of GPVI in WT mice increased the $FeCl_3$-induced occlusion time compared to IgG-treated controls, and GPVI inhibition alone had an even more severe effect on coagulation than Reelin absence in reeler mice[136]. Together along with positive coimmunoprecipitation data between GPVI and Reelin[136], this suggests Reelin as a new ligand for GPVI and shows that arterial thrombosis could be prevented with inhibition of Reelin production by platelets. These findings could be applied to a range of cardiovascular diseases including stroke, myocardial infarction, or aortic aneurysm formation.

Reelin increases platelet adhesion directly influencing coagulation.

Reelin promotes adhesion both in endothelial cells and platelets, hinting at a common mechanism between cell types. Overall, these coagulation studies demonstrate that Reelin increases platelet adhesion, and thus coagulation, but that there are multiple possible

mechanisms involved. It appears that increased platelet adhesion via Reelin is guided by various receptors, with APP and ApoER2 influencing fibrinogen adhesion and GPVI influencing collagen adhesion. More studies need to be performed to determine Reelin's specific effect on these mechanisms.

8) REELIN AND LYMPHATIC CIRCULATION

Reelin is produced by lymphatic endothelial cells and appears pertinent for lymphatic system development[82,138]. This is similar to how Reelin guides neuronal migration in brain development. However, unlike in the brain, studies have found that canonical Reelin signaling through Dab1 does not influence lymphatic development or circulation, promoting investigation of brand-new mechanisms. Interestingly, Reelin influences multiple cell-to-cell communications between lymphatic endothelial cells and varying muscle cells.

Reelin guides lymphatic vessel formation through communication between lymphatic endothelial cells and smooth muscle cells.

The lymphatic vasculature is composed of lymphatic capillaries, responsible for the intake of lymph, and collecting lymphatic vessels, responsible for lymph propulsion to lymph nodes and where Reelin is secreted by lymphatic endothelial cells[91,139]. Interestingly, not only do reeler mice show deficits in neuronal formation, but they have defective dermal lymphatic vessel formation and function, specifically related to collecting vessels, and a reduced amount of smooth muscle cell contractions[91]. Upon secretion by endothelial cells, Reelin's proteolytic processing is controlled by adjacent smooth muscle cells resulting in the proteolytic cleavage of the N terminus to form a 180 kDa fragment as well as the central Reelin fragment responsible for canonical Reelin signaling[91]. However, canonical Reelin signaling does not influence lymphatic development, proven by studying *Apoer2/Vldlr* and *Dab1* mutant mice, which showed normal lymphatic vessel formation[91]. Thus, either the central Reelin fragment acts through an unknown pathway or the 180 kDa fragment is the active form of Reelin in the lymphatic system. One possibility involves monocyte chemoattractant protein-1 (MCP-1) which is associated with the recruitment of mural cells (smooth muscle cells and pericytes) to endothelial cells[91,140–142]. Increased MCP-1 mRNA expression upon treatment with the central Reelin fragment and fulllength Reelin implicate its possible participation in Reelin signaling[91]. These novel findings are some of the first data to implicate smooth muscle cells in formation and regulation of the lymphatic system.

Reelin influences lymphatic endothelial cell and cardiomyocyte communication in cardiac lymphangiogenesis

Formation of new lymphatics, known as lymphangiogenesis, may prove beneficial to the injured heart by influencing immune cell clearance and cardiac repair as seen in multiple animal models[143,144]. Upon conditional deletion of the *Prox1* gene, an essential regulator of cardiac lymphangiogenesis[145], from murine lymphatic endothelial cells, mutant embryos had significantly smaller hearts with edema and lacked nearly all cardiac lymphatics when compared to controls at E17.5[146]. At a cellular level, cardiomyocyte production, proliferation, and apoptosis were significantly altered[146]. The authors observed that Reelin mRNA expression in lymphatic endothelial cells was greatly reduced by 80% in *Prox1*

mutant hearts. Similarly, conditional deletion of Reelin from lymphatic endothelial cells in mice caused mutants to develop smaller hearts and altered cardiomyocyte production and apoptosis[146]. Thus, Reelin has some importance in cardiac development; however, no cardiac alterations have been reported in reeler mice to date. Reelin increased phosphorylation of FAK, Dab1, AKT, and ERK via integrin-β1 in cultured primary cardiomyocytes treated with WT lymphatic endothelial cell-conditioned medium, but this did not occur when treating the cardiomyocytes with conditioned medium from *RELN*-deficient lymphatic endothelial cells[146]. This data insinuates that lymphatic endothelial cell and cardiomyocyte communication is needed but not critical for effective cardiac lymphatic development. Reelin influences this communication during embryonic development, yet the pathway signaling between Prox1 and Reelin remains to be explored.

Reelin promotes early postnatal cardiac repair.

Normal postnatal cardiac Reelin expression is reduced at P2 and steadily declines until it is barely detectable at P14[146]. After neonatal myocardial infarction of wild-type mice at P2, analysis at P7 showed that Reelin expression was re-activated in the infarcted hearts with higher expression in infarcted tissue[146]. When inducing myocardial infarction to both wild-type mice and reelers, the reeler mice had increased cardiac fibrosis and depressed cardiac function at P21[146]. Delivery of Reelin directly to the heart via patches surgically sutured to myocardial infarcted hearts in two-month old mice significantly improved cardiac function 21–42 days after myocardial infarction, reduced fibrotic scarring of the myocardium, and reduced cardiomyocyte apoptosis when compared to controls[146]. These results indicate that Reelin mediates and bolsters cardiac repair beyond embryonic stages and warrants additional studies in adult mice.

9) REELIN AND IMMUNE SYSTEM

It has been demonstrated that Reelin depletion decreases the recruitment of circulating leukocytes into different tissues due to decreased adhesion properties of the vascular endothelium. This result raises another question regarding Reelin's influence on immune cells. To date, no direct effect of Reelin has been reported on leukocytes[37,38,107]; however, a 30 years old study by Green-Johnson *et al.* suggested that lack of Reelin, during development only, may have a secondary effect on leukocytes. On one hand, they observed that reeler mice have a notable change in their cytokine profiles compared to homozygous WT or heterozygous littermates, with reduced expression of IL-1, IL-2, IL-4, and IFN-γ[147]. On the other hand, reelers have a higher concentration of norepinephrine due to altered brain development[147,148]. This abnormally high concentration of norepinephrine is caused by a reduction in cerebellar size without a compensating reduction of norepinephrine[148]. Knowing that increased norepinephrine levels directly altered cytokine production and macrophage count, it has been suspected by the authors that increased norepinephrine level in reeler mice have a secondary effect on leukocytes. Importantly for anti-Reelin therapies, this same effect would not be recapitulated in conditional or pharmacological knockouts of Reelin as these experiments would deplete Reelin after normal embryonic brain development has concluded. Indeed, white blood cell subpopulation counts are similar in WT and Reelin cKO mice[38].

10) REELIN AND THE INTESTINE

Due to the constant self-renewal process of intestinal cells, equilibrium between cell proliferation, differentiation into subclasses (colonocytes, goblet cells, enteroendocrine cells, and Paneth cells), and apoptosis is of key importance[149]. Reeler mice have more severe consequences after colonic injury indicating Reelin's protective involvement in the intestine.

Reelin expression is restricted to myofibroblasts and the muscle layer in mouse small intestine and colon[149]. Reelin receptors (ApoER2 and VLDLR) and Dab1 are expressed in both myofibroblasts and epithelial cells, namely colonocytes[149]. Myofibroblasts are characterized by their expression of α-smooth muscle actin (α-SMA) and are important to the intestine when regarding activation of immune response, inflammation (i.e. inflammatory bowel disease (IBD)), and epithelial regulation[150,151]. Dextran sulphate sodium (DSS)-induced colitis in mice produced various detrimental symptoms and vastly upregulated ApoER2, Reelin, and Dab1 with no change in VLDLR expression and minimal change in α-SMA, indicating that Reelin itself is upregulated and not myofibroblast cell production[149]. Reelin protein expression increased in the mucosa directly with duration of DSS treatment, further providing evidence that DSS-induced colitis activates Reelin gene expression[149]. When comparing DSS treatment of wild-type and reeler mice, more severe colitis progression was seen in the reeler phenotype characterized by higher disease activity index, higher mortality (40% vs. 10% in controls) and higher pro-inflammatory cytokine IL-1β expression[149]. Thus, the authors suggested that Reelin has a protective effect against DSS-induced colitis; however, this effect could be secondary to altered immune cell function as observed in reeler mice (see Reelin and Immune System). Further investigations using Reelin cKO or depletion in this model would be required to conclusively address this question.

Nevertheless, it appears that canonical Reelin signaling is active in the intestine and colon. Further study of the Reelin receptor ApoER2 and its intracellular adaptor protein Dab1 could provide valuable information on Reelin's influence in this organ's pathophysiology with important application for chronic inflammation in human bowel diseases such as Crohn's Disease.

11) REELIN AND THE KIDNEY

Although not essential, Reelin is expressed in the kidney during fetal and postnatal stages in lymphatic vessels[82] but also shows variable expression in specific kidney cells as detailed in this section. Reelin's expression is heightened during early fetal stages in humans and gradually decreases in late fetal stages, similar to human neuronal expression[92]. This trend is consistent with the conclusion of nephrogenesis at 38 weeks development, indicating Reelin's importance in early kidney formation[92]. In adults, increased Reelin expression has been observed in response to angiotensin II during kidney disease [152].

Reelin and Dab1 have differential protein expression in the kidney.

During fetal development, Reelin is detected in the cytoplasm of distal convoluted tubules (DCT), glomeruli, and is strongly expressed in proximal convoluted tubules (PCT)[92]. This

expression is nearly completely ablated in the postnatal kidney. At this time point, Reelin was only detected in PCT structures and glomeruli, albeit at much lower levels than during all embryonic time points. Dab1, a Reelin cytoplasmic adaptor protein, is expressed across all prenatal and postnatal stages in the DCT and glomeruli, with variable expression in the PCT[92]. Unlike Reelin, the highest expression of Dab1 is seen in the DCT throughout embryonic development[92]. During human postnatal periods at 1.5 years and 7 years of age, when nephrogenesis is fully complete and differentiation of nephrons occurs, overall Dab1 expression is much higher than Reelin expression, especially in the PCT[92]. The hypothesis for consistently high Dab1 expression is that this protein may trigger downstream pathways of ion exchange protein channels, helping the development and differentiation of nephrons in the kidney tissue. Dab1 and Reelin only have coexpression in the healthy human kidney during the early fetal stages, essentially disappearing upon birth[92]. Thus, another pathway (such as those involving Src family kinases) may be sufficient to trigger Dab1 in the absence of Reelin during kidney maturation and maintenance. These results need to be corroborated with other experimental methods, such as western blotting and RT-PCR, owing to the heightened autofluorescence of the kidney[153].

Reelin production is upregulated by Angiotensin II in kidney disease.

It has been suggested that podocytes play an important function in glomerular basement membrane morphogenesis during embryonic development of the kidney, as well as throughout the life of the organism in glomerular filtration[154,155]. Angiotensin II has been shown to increase podocyte apoptosis in both *in vivo* and *in vitro* models[152,156,157]. Podocyte apoptosis and excessive angiotensin II activation, through the renin-angiotensin system, lead to increased oxidative stress, activation of proinflammatory cytokines, and increased glomerular pressure ultimately resulting in kidney failure[158–160]. Angiotensin II increased pDab1 and Reelin levels as seen in Ang II-treated rats and cultured podocytes[152]. Further investigation of cellular proliferation and apoptosis via MAP kinase pathways showed that Dab1 siRNA diminished the response of the p38 pathway but not the ERK pathway following angiotensin II infusion[152]. This indicates that angiotensin II-induced podocyte apoptosis acts through phosphorylation of p38[152]. Thus, this hormone promotes Reelin expression and Dab1 phosphorylation in podocytes, suggesting Reelin's involvement in the progression of kidney disease.

Reelin is important for embryonic organ development in various tissues.

Reelin appears to have a ubiquitously important role in the development of the intestine, immune system, and kidney. However, Reelin also has a negative role as it bolsters the progression of postnatal disease. This is the case regardless of signaling pathway, either canonical or non-canonical. With the study of genetic or pharmacological depletion of Reelin, we can begin to uncover the importance, or lack thereof, of postnatal Reelin in the pathophysiology of various diseases such as kidney failure, IBD, or myocardial ischemia.

12) REELIN AND CANCER

Literature on Reelin and cancer is abundant and exposes a complex role for this extracellular protein in cancer development. Due to Reelin's roles in cell migration and

proliferation[161,162], conflicting data is seen on whether it is helpful or hindering to tumorigenesis (Table 1). One example is in neuroblastoma, a pediatric cancer of the nervous system, Reelin expression and cancer cell migration varied across different neuroblastoma cell lines[163]. Further, Reelin's involvement in individual cancers may make Reelin levels a helpful marker for cancer progression or initiation.

Cases where increased Reelin expression is associated with cancer progression.

In some cancers, Reelin upregulation directly relates to cancer prognosis and severity of disease. Multiple myeloma patients with low Reelin expression had better overall and event-free survival than those with high expression[162]. Reelin specifically was found to exacerbate symptoms in multiple myeloma patients due to enhanced aerobic glycolysis, increased cell adhesion, and overall enhanced survival of multiple myeloma cells[162,164]. Mechanistically, Reelin activates integrin β1 through the phosphorylation cascade FAK/Syk, PI3K/Akt/mTor, and STAT3. This cascade promotes transcription of cell-cycle genes, thus producing multiple signals towards cellular proliferation[162,164]. In this context, Reelin ablation has a protective effect against multiple myeloma by preventing cellular adhesion and decreasing proliferation of multiple myeloma cells.

Reelin expression was also found specifically in cancerous tissue in 39% of samples when looking at 66 cases of prostate cancers[165]. Within this sample set, no Reelin expression was found for prostate intraepithelial neoplasia lesions, a precursor to prostate cancer. Thus, Reelin levels could be a useful identifier of prostate cancer progression as Gleason score (a sum of primary and secondary patterns of cancerous cells) does not fully grasp the severity of disease[166].

Reelin was also found to induce cisplatin resistance in non-small cell lung cancer which accounts for ~84% of all lung cancer[167]. Cisplatin is a chemotherapeutic drug that halts DNA replication, causing eventual cell death, via the formation of DNA lesions[168]. Unfortunately, cancer patients can form cisplatin resistance causing limitations in treatment[168,169]. When looking at specialized cisplatin-resistant A549 lung cancer cells transfected with Reelin or control siRNA, Reelin suppression resulted in reduced cell viability and increased apoptosis when compared to controls[170]. Conversely, Reelin upregulation directly enhanced cisplatin resistance across various lung cancer cell lines, including two non-small cell lung cancer cell lines H1299 and H460, significantly improving cancer cell viability[170]. Reelin also promotes epithelial-mesenchymal transition (EMT) and thus cell migration of non-small cell lung cancer cells through activation of p38/GSK3β signaling[170]. Further, Reelin knockdown resulted in reduction of p38 and GSK3β phosphorylation in non-small cell lung cancer cells[170]. Thus, Reelin ablation could assist in chemotherapeutic treatment by reducing cisplatin resistance in lung cancer patients. Another cancer where Reelin is associated with chemoresistance is noted in acute myeloid leukemia, where mutations in the *RELN* gene are seen in pediatric and adult patients[171].

Cases where decreased Reelin expression is associated with cancer migration and proliferation.

While some cancers show increased Reelin expression in malignant cells as presented in the previous section, others present an extinction of Reelin expression and hypermethylation of the *RELN* gene. For example in breast cancer, Reelin expression is lost or strongly reduced in cancerous lesions compared to healthy tissue, with majority of lesions showing some degree of promoter methylation[172]. Upon transfection of a triple negative breast cancer (TNBC) cell line (MDA-MB 231) with a Reelin vector restoring expression, cell migration and invasion were mitigated[172]. In accordance with this, suppression of endogenous Reelin in another TNBC cell line, SUM159, increased the cell line's invasive potential[173]. Interestingly, when ablating integrin α3β1 in MDA-MB 231 cells through RNAi-mediated knockdown, Reelin mRNA significantly increased[173]. This gives insight into Reelin's role in tumor cell migration inhibition with integrin α3β1 as the responsible receptor for the spread of breast cancer cells via the downregulation of Reelin.

The epigenetic suppression of Reelin expression is reported in multiple types of cancers. When looking at normal colon and colorectal adenocarcinoma samples from the same patient, Reelin mRNA expression is downregulated due to an upregulation of DNMT1 and thus hypermethylation of the *RELN* promoter[174]. A lack of Reelin in the small intestine alters cell proliferation, migration and apoptosis, impairing the intestinal barrier and increasing the occurrence of colitis-associated tumorigenesis as seen in reeler mice[175,176]. Further, ApoER2 mRNA expression was upregulated, and the tumor suppressor gene *HIC1*, hypermethylated in cancer 1, had reduced mRNA expression in colonic adenocarcinoma samples[174].

Regarding pancreatic cancer, absent expression of Reelin protein and mRNA is seen across multiple types of pancreatic cancer tissues, and the majority of pancreatic cancer cell lines showed hypermethylation of both the *RELN* and *DAB1* genes, indicating a deficiency in Reelin signaling in this type of cancer[177]. In this pathology as well the loss of Reelin results in greatly enhanced cell motility, invasiveness, and colony-forming ability[177].

Lastly, hepatocellular carcinoma, a common form of liver cancer, demonstrates silencing of the *RELN* gene allowing for cellular migration and increasing the probability of metastasis[178]. A key factor in hepatocellular carcinoma severity is initiation of metastasis through EMT, granting epithelial cells the ability to invade other tissues due to lessened adherence[179]. Transforming growth factor-β (TGF-β), a major metabolic regulator of the TGF-β superfamily[180–184] known to activate EMT markers such as N-cadherin and vimentin[178,185,186], also inhibits Reelin expression. Furthermore, Reelin overexpression impairs TGF-β1-induced EMT enhancement supporting Reelin's importance in liver cancer prevention[178].

Whether protective or adverse, Reelin affects adhesion, migration, and proliferation of cancer cells.

These studies highlight the complexity of Reelin's role in cancer and suggest ambiguous functions either promoting or inhibiting malignant cell adhesion, migration and proliferation

depending on the cell type and organ affected. In this context, Reelin may appear as a specific biomarker for some types of tumors, such as multiple myeloma or prostate cancer. Reelin supplementation could also be a possible therapy for cancers exhibiting Reelin downregulation. Similarly, Reelin inhibition could be envisaged for some well-defined cancers, but would require a refined benefit / risk evaluation as systemic Reelin depletion may also affect the recruitment of leukocytes potentially fighting malignant cells. The heterogeneity of Reelin function in cancer types makes it difficult to conclude on a general mechanism. For example, while decreased Reelin expression is associated with increased cancer migration and proliferation in several cases, for the glioblastoma cell lines U251 and U87 the opposite association was observed[187,188].

Finally, a switch in Reelin source may explain different and conflicting functions as it has been suggested that Reelin has a dual role in neuroblastoma formation. Autocrine expression marks low-grade differentiating tumor cells, whereas paracrine Reelin presented by lymphatic and blood vessel endothelium may act as a chemoattractant and promote hematogenic and lymphogenic dissemination in progressed stages[163].

13) FUTURE PERSPECTIVES AND CONCLUSION

As presented in this review, studies are emerging on Reelin's importance in various systems and diseases outside of the central nervous system. Our understanding of this extracellular protein's biology is still growing and mostly leans on knowledge gained through neuronal research in addition to the extended use of reeler mice. However, this model has major disadvantages for scientific investigations, being limited by brain malformation and immune system aberrations. Specifically, altered brain development in these animals lead to increased norepinephrine expression that affects cytokine production, as well as macrophage and lymphocyte composition[147]. Instead, the use of Reelin cKO mice is more appropriate, with an induction of the KO phenotype in adult mice after proper brain development. In addition to this genetic mouse model, different tools have been developed to pharmacologically deplete Reelin, such as the commercially available monoclonal anti-Reelin antibody CR-50 created by Dr. Mikoshiba in 1995, or antisense oligonucleotides (ASO) against Reelin[7,27,37,97]. CR-50 works by binding the N-terminal part of Reelin thus preventing its multimerization[27]. In addition, intraperitoneal injections of CR-50 in mice lead to an immune complex formation with which rapidly clears this protein from the circulation[38]. Importantly, peripheral anti-Reelin treatment has not shown a depletion of Reelin in the brain, thus preserving its function in neuronal activity while abrogating its possibly deleterious effect outside of the CNS.

Most of the studies so far have focused on Reelin's separate effect in each system. However, different Reelin functions may have cumulative effects. For example, in the circulation, depletion of Reelin decreases coagulation, yet in the endothelium, Reelin reduction leads to decreased leukocyte adhesion and increased eNOS activity. Altogether, these three functions of Reelin could participate synergistically, yielding superior organ perfusion and reduction of inflammation.

Some important questions remain unclear, probably due to the multitude and complex Reelin functions in different organs. For one, Reelin is abundant in the plasma; however, its major cellular source and its regulation within an organism remains unknown. Outside of the CNS, Reelin expression has been detected in the liver, kidney, adrenal medulla, lymphatic vessels, small intestine, submandibular gland, cartilage, and bone[83,90–96]. Intriguingly, circulating Reelin increases in different inflammatory conditions such as MS and atherosclerosis. Reelin has also been observed in lymphatic endothelial cells, human umbilical vein endothelial cells, and human microvascular endothelial cells. Beside its source, how Reelin is induced, regulated, and how its structure or cleavage affect its functions still remains elusive. Answering these questions would fill a gap in our current knowledge of Reelin biology with profound implication for different pathologies involving this protein.

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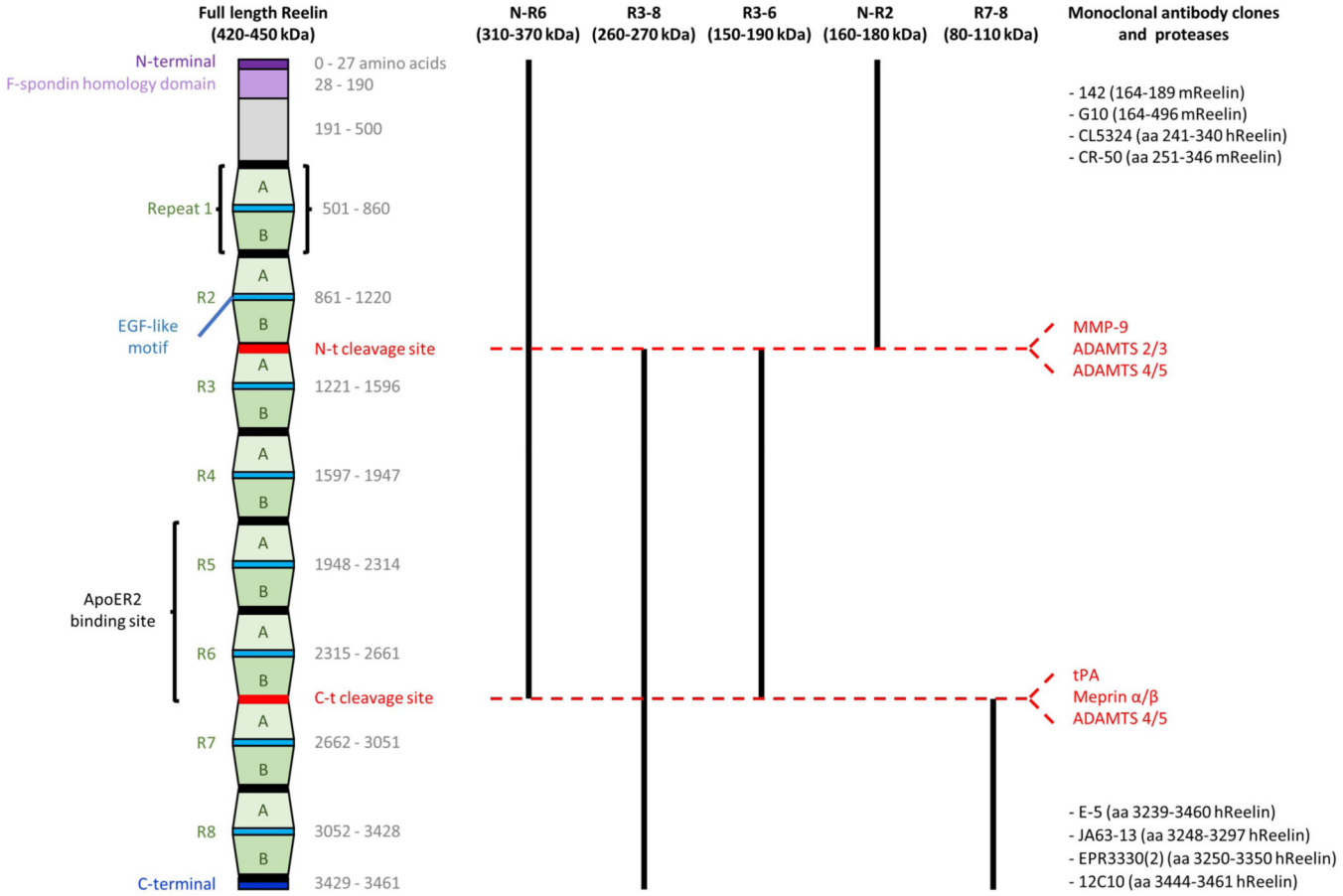

Figure 1. Reelin structure, cleavage products, interaction with proteases and antibody binding sites.

Reelin is a large extracellular protein with many glycosylation sites affecting its molecular weight and two major cleavage site generating multiple cleavage products.

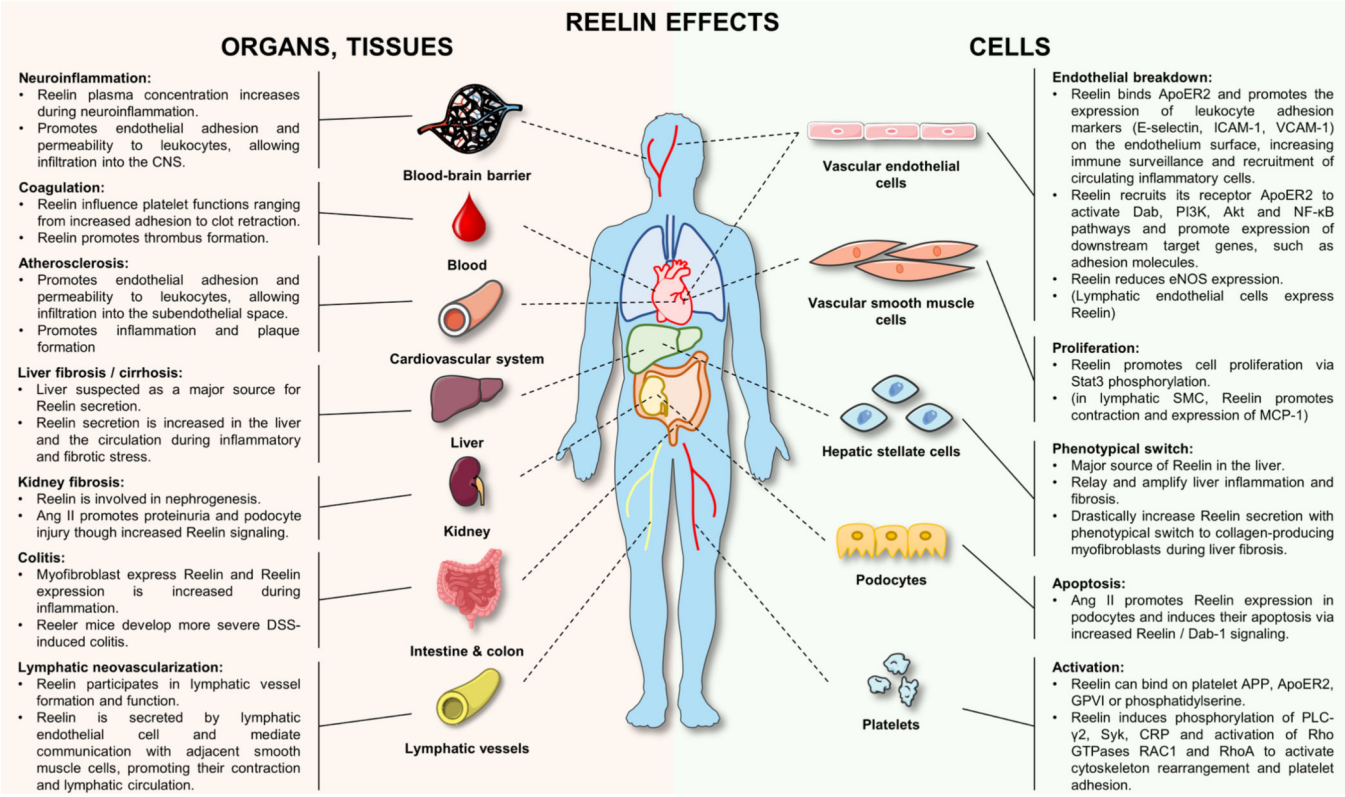

Figure 2. Overview of Reelin expression and function among organs and cell types.

Besides neurological function, Reelin is secreted by different cells and is present in many organs mainly linked to the cardiovascular system, where it regulates organ development, vascular permeability, inflammation, fibrosis and thrombosis among others.

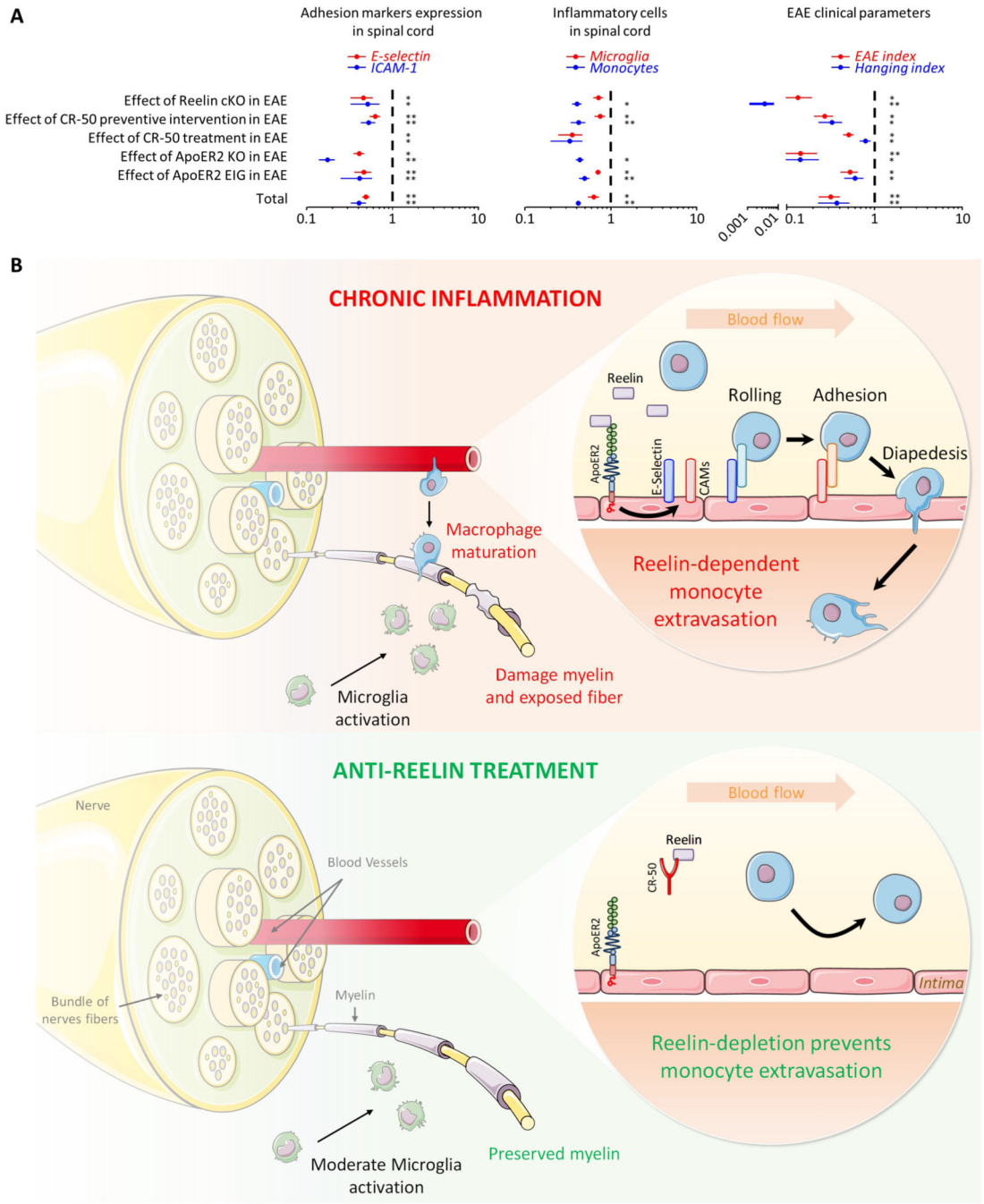

Figure 3. Inhibition of Reelin pathway protects from neuroinflammation.

(**A**) Meta-analysis on all the literature available [37,107] on Reelin or Apoer2 inhibition in experimental autoimmune encephalomyelitis (EAE) mouse models. Expression of adhesion markers were evaluated by Western blotting and the presence of inflammatory cells by immunohistochemistry or FACS, all normalized to corresponding controls. EAE severity was evaluated daily from day 10 by EAE clinical score (from 0 = unaffected to 10 = dead) and hanging test (for a maximum time of 180 s). EAE severity and hanging time indexes were calculated for each animal by integrating daily scores over the course of the

experiment and represent the area under the curve (AUC). The graph represents the fold change (compared to controls set as 1, dotted line) on a logarithmic scale. *$p<0.05$ and **$p<0.01$ (t-test). (**B**) This mechanistic model incorporates the findings described in this article and the literature to date, as discussed. Reelin promotes the expression of vascular adhesion proteins, thereby increasing monocyte adhesion/extravasation, inflammation and consequently demyelination. Both genetic or therapeutic Reelin depletion prevents this inflammatory cascade and thus paralysis in a murine EAE model. We surmise that the same mechanism is conserved in humans where Reelin also promotes monocyte adhesion to vascular endothelial cells in vitro. Adapted from [37,107] with permission from the authors.

Table 1.

Reelin's Protective and Adverse Effects on Various Cancer Types

Type of Cancer	Reelin Upregulated or Downregulated?	Protective or Adverse Effect?	Summary of Findings	Associated proteins/ pathways	Reference
Multiple Myeloma	Upregulated	Adverse	Patients showed a significant negative correlation between RELN expression and patient outcome.	Integrin β1, HIF1α	162
Multiple Myeloma	Upregulated	Adverse	H929 and U266, human myeloma cell lines, showed increased adhesion of MM cells to BMSCs with increased Reelin concentration.	Integrin β1	189
Prostate	Upregulated	Adverse	Reelin immunohistochemistry staining of 66 cases of prostate cancer showed a direct association between cancer progression and reelin expression.		165
Lung	Upregulated	Adverse	Reelin upregulation increased cisplatin resistance in multiple lung cancer cell lines. Silencing Reelin production increased apoptosis among cisplatin-resistant A549 lung cancer cells.	p38, GSK3β	170
TNBC	Upregulated	Protective	MDA-MB 231 cells transfected to produce Reelin had less migratory and invasive cellular behavior. Reelin siRNA treatment of SUM159 cells causes increased invasive potential.	Integrin α3β1	173
Colorectal	Downregulated	Adverse	There is an increasing occurrence of colitis-associated tumorigenesis when comparing reeler mice to controls.	Sox9, Cdx2, Smad5, Cyclin D1	175
Colorectal	Downregulated	Adverse	Reelin depletion is a result of upregulated ApoER2 mRNA expression and HIC1 mRNA downregulation as seen in adenocarcinoma samples.	ApoER2, HIC1	174
Hepatocellular Carcinoma	Downregulated	Adverse	As a result of RELN inhibition by siRNAs or RELN depletion with TGF-β treatment, EMT increased, allowing for higher initiation of metastasis.	TGF-β1, N-cadherin, Vimentin, Fibronectin	178
Hepatocellular Carcinoma	Downregulated	Adverse	Hepatocellular carcinoma tumor tissue had reduced RNA Reelin expression in 70.8% of samples. 37.5% of these tissues had some degree of hypermethylation at the RELN promoter.		190
Pancreatic	Downregulated	Adverse	Su8686 pancreatic cancer cell line had increased cell motility upon Reelin silencing. Absence of RELN mRNA expression seen in 18 different pancreatic cell lines.		177
Breast Cancer	Downregulated	Adverse	Reduced expression of Reelin is a result of promoter methylation.		172

MM: multiple myeloma, BMSC: bone marrow stromal cells